

**CLAIMS**

**The invention is claimed as follows:**

1. An adchromosomal plant comprising a mini-chromosome, wherein said mini-chromosome has a transmission efficiency during mitotic division of at least 90%.
2. The plant according to claim 1, wherein the mini-chromosome has a transmission efficiency during mitotic division of at least 95%.
3. The plant according to claim 1 or 2, wherein the mini-chromosome has a transmission efficiency during meiotic division of at least 80%.
4. The plant according to claim 3, wherein the mini-chromosome has a transmission efficiency during meiotic division of at least 85%.
5. The plant according to claim 4, wherein the mini-chromosome has a transmission efficiency during meiotic division of at least 90%.
6. The plant according to claim 5, wherein the mini-chromosome has a transmission efficiency during meiotic division of at least 95%.
7. The plant according to any one of claims 1-6, wherein the mini-chromosome is 1000 kilobases or less in length.
8. The plant according to claim 7 wherein the mini-chromosome is 600 kilobases or less in length.
9. The plant according to claim 8 wherein the mini-chromosome is 500 kilobases or less in length.
10. The plant according to any one of claims 1-9, wherein the mini-chromosome comprises a site for site-specific recombination.

11. The plant according to any one of claims 1-10, wherein the mini-chromosome comprises a centromeric nucleic acid insert derived from a crop plant centromere.

12. The plant according to claim 11, wherein the centromeric nucleic acid insert is derived from genomic DNA of a plant selected from the group consisting of *Brassica*, *Nicotiana*, *Lycopersicum*, *Glycine* or *Zea* species.

13. The plant according to claim 12, wherein the centromeric nucleic acid insert is derived from genomic DNA of a plant selected from the group consisting of broccoli, canola, tobacco, tomato, soybean or corn.

14. The plant according to any one of claims 1-13, wherein the mini-chromosome comprises a centromeric nucleic acid insert that comprises artificially synthesized repeated nucleotide sequences.

15. The plant according to any one of claims 1-14, wherein the mini-chromosome is derived from a donor clone or a centromere clone and has substitutions, deletions, insertions, duplications or arrangements of one or more nucleotides in the mini-chromosome compared to the nucleotide sequence of the donor clone or centromere clone.

16. The plant of any one of claims 1-15, wherein the mini-chromosome is obtained by passage of the mini-chromosome through one or more hosts.

17. The plant of Claim 16, wherein the mini-chromosome is obtained by passage of the mini-chromosome through two or more different hosts.

18. The plant of Claim 17, wherein the host is selected from the group consisting of viruses, bacteria, yeasts, plants, prokaryotic organisms, or eukaryotic organisms.

19. The plant according to any one of claims 1-18 wherein the mini-chromosome comprises one or more exogenous nucleic acids.
20. The plant according to claim 19, wherein the mini-chromosome comprises at least two or more exogenous nucleic acids.
21. The plant according to claim 20, wherein the mini-chromosome comprises at least three or more exogenous nucleic acids.
22. The plant according to claim 21, wherein the mini-chromosome comprises at least four or more exogenous nucleic acids.
23. The plant according to claim 22, wherein the mini-chromosome comprises at least five or more exogenous nucleic acids.
24. The plant according to claim 23, wherein the mini-chromosome comprises at least ten or more exogenous nucleic acids.
25. The plant according to any one of claims 19-24, wherein at least one exogenous nucleic acid is operably linked to a heterologous regulatory sequence functional in plant cells.
26. The plant according to claim 25, wherein the regulatory sequence is a plant regulatory sequence.
27. The plant according to claim 25, wherein the regulatory sequence is a non-plant regulatory sequence.
28. The plant according to claim 27, wherein the regulatory sequence is an insect or yeast regulatory sequence.

29. The plant according to claim 27, wherein the non-plant regulatory sequence comprises any one of SEQ ID NOS: 4 to 23 or a functional fragment thereof.

30. The plant according to any one of claims 1-29, wherein the mini-chromosome comprises an exogenous nucleic acid that confers herbicide resistance, insect resistance, disease resistance, or stress resistance on the plant.

31. The plant according to claim 30 wherein the exogenous nucleic acid confers resistance to phosphinothricin or glyphosate herbicide.

32. The plant according to claim 31 wherein the exogenous nucleic acid encodes a phosphinothricin acetyltransferase, glyphosate acetyltransferase or a mutant enolpyruvyl shikimate phosphate (EPSP) synthase.

33. The plant according to any one of claims 1-32, wherein the mini-chromosome comprises an exogenous nucleic acid that encodes a *Bacillus thuringiensis* crystal toxin gene or *Bacillus cereus* toxin gene.

34. The plant according to any one of claims 1-38, wherein the mini-chromosome comprises an exogenous nucleic acid that confers resistance to drought, heat, chilling, freezing, excessive moisture, ultraviolet light, ionizing radiation, mechanical stress, toxins, pollution, or salt stress.

35. The plant according to any one of claims 1-34, wherein the mini-chromosome comprises an exogenous nucleic acid that confers resistance to a virus, bacteria, fungi or nematode.

36. The plant according to any one of claims 1-35, wherein the mini-chromosome comprises an exogenous nucleic acid conferring herbicide resistance, an exogenous nucleic acid conferring insect resistance, and at least one additional exogenous nucleic acid.

37. The plant according to any one of claims 1-36, wherein the mini-chromosome comprises an exogenous nucleic acid is selected from the group consisting of a nitrogen fixation gene, a plant stress-induced gene, a nutrient utilization gene, a gene that affects plant pigmentation, a gene that encodes an antisense or ribozyme molecule, a gene encoding a secretable antigen, a toxin gene, a receptor gene, a ligand gene, a seed storage gene, a hormone gene, an enzyme gene, an interleukin gene, a clotting factor gene, a cytokine gene, an antibody gene, a growth factor gene, a transcription factor gene, a transcriptional repressor gene, a DNA-binding protein gene, a recombination gene, a DNA replication gene, a programmed cell death gene, a kinase gene, a phosphatase gene, a G protein gene, a cyclin gene, a cell cycle control gene, a gene involved in transcription, a gene involved in translation, a gene involved in RNA processing, a gene involved in RNAi, an organellar gene, a intracellular trafficking gene, an integral membrane protein gene, a transporter gene, a membrane channel protein gene, a cell wall gene, a gene involved in protein processing, a gene involved in protein modification, a gene involved in protein degradation, a gene involved in metabolism, a gene involved in biosynthesis, a gene involved in assimilation of nitrogen or other elements or nutrients, a gene involved in controlling carbon flux, gene involved in respiration, a gene involved in photosynthesis, a gene involved in light sensing, a gene involved in organogenesis, a gene involved in embryogenesis, a gene involved in differentiation, a gene involved in meiotic drive, a gene involved in self incompatibility, a gene involved in development, a gene involved in nutrient, metabolite or mineral transport, a gene involved in nutrient, metabolite or mineral storage, a calcium-binding protein gene, or a lipid-binding protein gene.

38. The plant according to claim 37, wherein the enzyme gene is selected from the group consisting of a gene that encodes an enzyme involved in metabolizing biochemical wastes for use in bioremediation, a gene that encodes an enzyme for modifying pathways that produce secondary plant metabolites, a gene that encodes an enzyme that produces a pharmaceutical, a gene that encodes an enzyme that improves changes the nutritional content of a plant, a gene that encodes an enzyme involved in vitamin synthesis, a gene that encodes an enzyme involved in carbohydrate, polysaccharide or starch synthesis, a gene that encodes an enzyme involved in mineral accumulation or availability, a gene that encodes a phytase, a

gene that encodes an enzyme involved in fatty acid, fat or oil synthesis, a gene that encodes an enzyme involved in synthesis of chemicals or plastics, a gene that encodes an enzyme involved in synthesis of a fuel and a gene that encodes an enzyme involved in synthesis of a fragrance, a gene that encodes an enzyme involved in synthesis of a flavor, a gene that encodes an enzyme involved in synthesis of a pigment or dye, a gene that encodes an enzyme involved in synthesis of a hydrocarbon, a gene that encodes an enzyme involved in synthesis of a structural or fibrous compound, a gene that encodes an enzyme involved in synthesis of a food additive, a gene that encodes an enzyme involved in synthesis of a chemical insecticide, a gene that encodes an enzyme involved in synthesis of an insect repellent, or a gene controlling carbon flux in a plant.

39. The plant according to any one of claims 37, wherein the centromere of the mini-chromosome comprises  $n$  copies of a repeated nucleotide sequence, wherein  $n$  is less than 1000.

40. The plant according to any one of claims 1-38, wherein the centromere of the mini-chromosome comprises  $n$  copies of a repeated nucleotide sequence, wherein  $n$  is at least 5.

41. The plant according to any one of claims 1-38, wherein the centromere of the mini-chromosome comprises  $n$  copies of a repeated nucleotide sequence, wherein  $n$  is at least 15.

42. The plant according to claim 41, wherein the centromere of the mini-chromosome comprises  $n$  copies of a repeated nucleotide sequence, wherein  $n$  is at least 50.

43. The plant according to any one of claims 1-42, wherein the mini-chromosome comprises a telomere.

44. The plant according to any one of claims 1-42, wherein the mini-chromosome is circular.

45. The plant according to any one of claims 1-44, wherein the plant is a monocotyledone.
46. The plant according to any one of claims 1-44, wherein the plant is a dicotyledone.
47. The plant according to any one of claims 1-44, wherein the plant is a cereal plant.
48. The plant according to any one of claims 1-44, wherein the plant is from the *Brassica*, *Nicotiana*, *Lycopersicum*, *Glycine* or *Zea* species.
49. The plant according to any one of claims 1-44, wherein the plant is a vegetable crop.
50. The plant according to any one of claims 1-44, wherein the plant is a field crop.
51. The plant according to any one of claims 1-44, where the plant is a fruit and vine crop.
52. The plant according to any one of claims 1-44, wherein the plant is wood or fiber crop.
53. The plant according to any one of claims 1-44, wherein the plant is an ornamental plant.
54. A part of the plant according to any one of claims 1-53.
55. The plant part according to claim 54 which is a pod, root, cutting, tuber, stem, stalk, fruit, berry, nut, flower, leaf, bark, wood, epidermis, vascular tissue, organ, protoplast, crown, callus culture, petiole, petal, sepal, stamen, stigma, style, bud, meristem, cambium, cortex, pith, sheath, silk or embryo.

56. A meiocyte or gamete or ovule or pollen or endosperm of the plant according to any one of claims 1-53.
57. A seed, embryo or propagule of the plant according to any one of claims 1-53.
58. A progeny of the plant according to any one of claims 1-53.
59. The progeny of claim 58 wherein the progeny is the result of self-breeding.
60. The progeny of claim 58 wherein the progeny is the result of cross-breeding.
61. The progeny of claim 58 wherein the progeny is the result of apomyxis.
62. The progeny of claim 58 wherein the progeny is the result of clonal propagation.
63. The progeny of claim 58 comprising a mini-chromosome descended from a parental mini-chromosome that contained a centromere less than 150 kilobases in length.
64. The progeny of claim 58 comprising a mini-chromosome descended from a parental mini-chromosome that contained a centromere less than 100 kilobases in length.
65. The progeny of claim 58 comprising a mini-chromosome descended from a parental mini-chromosome that contained a centromere less than 50 kilobases in length.
66. A method of making a mini-chromosome for use in the plant according to any one of claims 1-53 comprising



identifying a centromere nucleotide sequence in a genomic DNA library using a multiplicity of diverse probes, and  
constructing a mini-chromosome comprising the centromere nucleotide sequence.

67. The method of claim 66 wherein the identifying further comprises  
determining hybridization scores for hybridization of the multiplicity of diverse probes to genomic clones within the genomic DNA library,  
determining a classification for genomic clones within the genomic DNA library according to the hybridization scores for at least two of the diverse probes, and  
selecting one or more genomic clones within one or more classifications for constructing the mini-chromosome.

68. The method of claim 66 or 67 wherein at least three different probes are used.

69. The method of claim 68 wherein at least four different probes are used.

70. The method of claim 69 wherein at least five different probes are used.

71. The method of claim 70 wherein at least ten different probes are used.

72. The method of any one of claims 66-71 wherein at least one probe hybridizes to the centromere region of a chromosome.

73. The method of any one of claims 66-71 wherein at least one probe hybridizes to satellite repeat DNA.

74. The method of any one of claims 66-71 wherein at least one probe hybridizes to retroelement DNA.

75. The method of any one of claims 66-71 wherein at least one probe hybridizes to portions of genomic DNA that are heavily methylated.

76. The method of any one of claims 66-71 wherein at least one probe hybridizes to arrays of tandem repeats in genomic DNA.

77. The method of any one of claims 66-71 wherein at least one probe hybridizes to ribosomal DNA, and a classification comprises a low hybridization score for hybridization to said probe.

78. The method of any one of claims 66-71 wherein at least one probe hybridizes to mitochondrial DNA, and a classification comprises a low hybridization score for hybridization to said probe.

79. The method of any one of claims 66-71 wherein at least one probe hybridizes to chloroplast DNA, and a classification comprises a low hybridization score for hybridization to said probe.

80. The method of any one of claims 66-71 wherein at least one probe hybridizes to telomere DNA.

81. The method of any one of claims 66-71 wherein at least one probe hybridizes to a pseudogene.

82. A method of making a plant according to any one of claims 1-53 comprising

delivering a mini-chromosome to a plant cell using a biolistic method, wherein a particle suitable for use in a biolistic method is delivered in a liquid with the mini-chromosome, and

regenerating a plant from the plant cell.

83. The method of claim 82 wherein the liquid further comprises a divalent ion and a di- or poly-amine.

84. A method of making a plant according to any one of claims 1-53 comprising

co-delivering to a plant cell a mini-chromosome and a nucleic acid encoding a growth inducing gene, wherein said nucleic acid is not part of the mini-chromosome, and

regenerating a plant.

85. The method of claim 84 wherein the nucleic acid encoding a growth inducing gene is not expressed or not present in the regenerated plant.

86. The method of claim 84 wherein the nucleic acid encoding a growth inducing gene is expressed during regenerating the plant.

87. The method of any one of claims 84-86 wherein the growth inducing gene is selected from the group consisting of encoding plant growth regulator genes, organogenesis-promoting, embryogenesis-promoting or regeneration-promoting genes

88. The method of claim 87 wherein the gene is a *Agrobacterium tumefaciens* isopentenyl transferase gene, *Agrobacterium rhizogenes* isopentenyl transferase gene, *Agrobacterium tumefaciens* indole-3-acetamide hydrolase (IAAH) gene or *Agrobacterium tumefaciens* tryptophan-2-monooxygenase (IAAM) gene.

89. A method of using a plant according to any one of claims 1-53 to produce a food product comprising the steps of growing the plant, and harvesting or processing the plant.

90. A method of using a plant according to any one of claims 1-53 to produce a recombinant protein comprising the step of growing a plant comprising a

mini-chromosome that comprises an exogenous nucleic acid encoding the recombinant protein.

91. The method of claim 90 further comprising the steps of harvesting the plant and isolating the recombinant protein from the plant.

92. The method of claim 90 or 91 wherein the recombinant protein is a pharmaceutical protein.

93. A method of using a plant according to any one of claims 1-53 to produce a chemical product comprising the step of growing a plant comprising a mini-chromosome that comprises an exogenous nucleic acid encoding an enzyme involved in synthesis of the chemical product.

94. The method of claim 93 further comprising the steps of harvesting the plant and isolating the chemical product from the plant.

95. The method of claim 93 or 94 wherein the chemical product is a pharmaceutical product.